

**REMARKS**

Claims 1-109 are pending after entry of this paper. Claims 2-65, 80-83 and 109 remain rejected. Claims 67-79 and 84-108 have been withdrawn. Applicants reserve the right to pursue withdrawn claims in a divisional or continuing application. Applicants acknowledge the allowance of claim 1. In addition, applicants acknowledge that claim 66 has been objected to for being dependent from a rejected claim, but may be allowable if rewritten in the independent form.

Claims 2 and 4 have been amended to add the phrase “isolating composition having neutral and/or alkaline cellulase activity from the wild-type or mutant fungus” as suggested by the Examiner (Office Action – page 2). Support may be found throughout the instant specification and claims as originally filed.

No new matter has been introduced by these amendments. Reconsideration and withdrawal of the pending rejections in view of the above claim amendments and below remarks are respectfully requested.

**Response to Rejections under 35 U.S.C. §112, first paragraph**

Claims 2-65 and 80-83 stand rejected under 35 U.S.C. §112, first paragraph for allegedly failing to comply with the written description requirement. Specifically, the Examiner alleges while citing MPEP §2163 and *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406, that the instant application does not disclose a representative number of species to adequately describe the entire genus. Furthermore, the Examiner alleges that the instant specification does not disclose any structural features and properties commonly shared and possessed by each of the

claimed genus of cellulases, genus of endoglucanases and a genus of cellobiohydrolases (Office Action – page 3). The Examiner again reiterates that the scope of each genus includes many members with widely differing structural, chemical, and physicochemical properties such as, for example, differing amino acid sequences and that there is no correlation between these structures with their respective enzymatic activities. Thus, according to the Examiner, one skilled in the art cannot predict, visualize, and/or recognize the identity of the members of each claimed genus. Applicants respectfully disagree with the Examiner for the following reasons.

As an initial matter, applicants wish to remind the Examiner that “[e]ach claim must be separately analyzed and given its broadest reasonable interpretation in light of and consistent with the written description. See, e.g., *In re Morris*, 127 F.3d 1048, 1053-54, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997). The entire claim must be considered ...” (See, MPEP 2163(1)). Applicants wish to draw the Examiner’s attention to claims 2, 3, 5, 6, 25, 26, 31, 32, 37, 38, 41, 42, 44, 45, 47, 48, 50, 51, 53, 54, 56, 57, 59, 60, 62, 63, 65, 66 and 109. These claims are directed explicitly to the fungi examined and described in the specification, *i.e.*, *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, and *Chrysosporium tropicum*. Thus, applicants wish to emphasize that the instant application presents a description of an actual reduction to practice (see *Brunswick Corp. v. U.S.*, 34 Fed. Cl. 532, 584, 1995 for definition) and clearly demonstrates possession of the claimed invention. Support for this assertion may be found throughout the instant specification, in particular Examples 1-17. Specifically, Examples 1-3 of the instant specification describe how the strain of *Chrysosporium lucknowense* was isolated and characterized. Examples 4 and 5 demonstrate how a composition with cellulase activity of *Chrysosporium lucknowense* was prepared and tested.

Various species of the same genera, *Chrysosporium*, were tested for cellulase activity in Example 8. These species include *Chrysosporium lucknowense* (ATCC-44006), *Chrysosporium pannorum* (ATCC-34151), *Chrysosporium pruinosa* (ATCC-24782), *Chrysosporium keratinophilum* (VKMF-2119 and VKMF-2875), *Chrysosporium lobatum* (VKMF-2120), *Chrysosporium merdarium* (VKMF-2121), *Chrysosporium queenslandicum* (VKMF-2116 and VKMF-2117) and *Chrysosporium tropicum* (VKMF-2877). Such extensive actual reduction to practice clearly demonstrates that the applicant had possession of the claimed invention and for that reason satisfies the written description requirement under 35 U.S.C. §112, first paragraph since the demonstration of actual reduction to practice is considered “safe harbor” under the MPEP guidelines.

Similarly, these arguments are partially applicable to claims 7-23, 27, 28, 29 and 39, which are dependant from the above recited claims. The Examiner is invited to provide any support to his contention that the species of *Chrysosporium* genus described in these claims for these particular seven species of *Chrysosporium* genus are not disclosed adequately in the specification as filed, since the specification presents evidence of actual reduction to practice as shown in Examples 1-17, particularly Example 8.

Moreover, there is no need to unduly burden the applicant, the Patent Office and the public with the well characterized *Chrysosporium* genus. Examples demonstrating methods of isolating, characterizing, and measuring cellulase activity are found on pages 20-30, where Example 1 describes the isolation of a strain (C1) from a exemplary sample, the characterization of the C1 strain in Examples 2-3, and enzyme assays from the Materials and Methods section and Example 4. The skilled artisan, reading the instant specification and based on what was

commonly known and understood in the art at the time the application was filed, would understand that the applicant had possession of the claimed invention.

The Examiner contends that “there [are] no disclosed structural features and properties commonly shared and possessed by each of the claimed [genus of cellulases].” (Office Action – page 3, second paragraph) and “the specification does not describe and define any structural features and amino acid sequences commonly possessed by each genus. [sic] Thus, one skilled in the art cannot visualize or recognize the identity of the members of each claimed genus” (Office Action – page 3, third paragraph). Applicants respectfully disagree and wish to draw the Examiner’s attention to MPEP §2163 that clearly states “there is no per se rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure” *Falkner v. Inglis*, 448 F.3d 1357, 1366, 79 USPQ2d 1001, 1007 (Fed. Cir. 2006). Therefore, applicants assert that the structure need not be recited in order to present an adequate written description.

Furthermore, as noted by the Examiner “the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice [sic] by disclosure of [sic] structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that applicant was in possession of the claimed genus. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406” (Office Action – page 3).

For example, claim 7 considered as a whole is directed to a genus of cellulase enzymes. The specification does disclose seven (7) cellulase species produced by different strains of *Chrysosporium* (See Example 8) which are identified by multiple relevant identifying

characteristics such as, for example, (1) substrate specificity, *i.e.*, ability to hydrolyze the cellulose at  $\beta$ -1,4-glucan linkages, (2) optimum ranges of temperature, *i.e.*, from about 40° C to about 60° C, and (3) pH, *i.e.*, from about 5.0 to about 11.0, for the catalytic activity, effects of chelating agents and proteases. Contrary to the Examiner's contention, the art of enzymology is well-developed (*Catalysis in Chemistry and Enzymology*, W.P. Jencks, Dover Publications, New York, 1987) and these identifying characteristics are sufficient for a skilled artisan to recognize that the applicants had possession of the species from the identifying characteristics of the seven cellulase species. In so doing, identifying characteristics of the other members of the genus may reasonably be predicted, thus, readily establishing possession of the genus (see Linton, et al., *The Journal of Experimental Biology* 207, 4095-4104, 2004; previously submitted).

With respect to the Examiner's contention that cellulases, endoglucanases and cellobiohydrolases are separate genera (Office Action – page 3), applicants again assert that the art recognizes one genus of cellulases (See Response to an Office Action filed June 6, 2007). Therefore, endoglucanases and cellobiohydrolases should be considered as further subgroups of cellulases and should not be treated as separate genera. Cellulase compositions are commonly known and understood in the art as having several different enzyme components, including those identified as exocellobiohydrolases, endoglucanases, and  $\beta$ -glucosidases. The complete cellulase system is required to efficiently convert crystalline cellulose to glucose. Generally, if total hydrolysis of a cellulose substrate is needed, the cellulase mixture should contain  $\beta$ -glucosidases and cellobiohydrolases, as well as endoglucanases. Therefore, in the variety of industrial textile applications, *e.g.*, stone washing, the use of a cellulase mixture would be desirable, which is clearly described in the claimed invention.

Thus, one skilled in the art could easily predict and/or recognize the identity of the members of the cellulase family by employing fungus from the *Chrysosporium* genus and isolating a composition with cellulase activity as described in the claimed invention. For the reasons outlined above, applicants assert that the subject of the instant application is fully enabled and complies with the written description requirement. Reconsideration and withdrawal of the written description rejection under 35 U.S.C. §112, first paragraph are respectfully requested.

Response to Rejections under 35 U.S.C. §112, second paragraph

Claims 2-23 and 109 have been rejected under 35 U.S.C. §112, second paragraph for indefiniteness. Specifically, the Examiner alleges that it is not known how the compositions can be made by growing any of the recited fungi (Office Action – page 2). Applicants respectfully disagree.

However, in order to expedite prosecution and without disclaimer of or prejudice to the subject matter recited therein, applicants have amended claims 2 and 4 to incorporate the step of isolating the composition having neutral and/or alkaline cellulase activity from the wild-type or mutant fungus as suggested by the Examiner (Office Action – page 2). Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §112, second paragraph rejection of claims 2-23 and 109 for indefiniteness.

Response to Rejections under 35 U.S.C. §103

Claims 2-4, 6-65, 80-83 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Parslow, et al. (U.S. Patent 4,661,289) in view of Janeckova, et al. (*Ceska Mykologie* 31(4):206-213, 1977). According to the Examiner, Parslow allegedly teaches compositions comprising fungal cellulase, surfactants, cationic fabric-softening compound, and builders, except it does not teach the claimed composition comprising fungal cellulase from the genus *Chrysosporium*, in particular *Chrysosporium lucknowense*. The Examiner alleges that Janeckova teaches *Chrysosporium lucknowense* isolated from soil. To support the case of *prima facie* obviousness, the Examiner point to the Bukhtojarov, et al. publication (*Biochemistry (Mosc)* 69(5):542-551, 2004) which allegedly provides evidence that *Chrysosporium lucknowense* contains cellulolytic enzymes including endoglucanases, cellobiohydrolases and cellulases that have neutral and/or alkaline cellulose activity (referring to EG24 and EG47), it would have been obvious to one skilled in the art at the time of the invention to modify teachings of Parslow to utilize cellulolytic enzymes from *Chrysosporium lucknowense* isolated by Janeckova (Office Action – page 4). Applicants respectfully disagree.

Applicants assert that contrary to the Examiner's contention, the claimed invention is not made obvious by Parslow in view of Janeckova. The Examiner admits that Parslow does not teach the claimed composition comprising fungal cellulase from genus *Chrysosporum*, in particular *Chrysosporium lucknowense* (Office Action – page 4). However, applicants respectfully wish to highlight that neither does Janeckova teach the cellulolytic enzymes isolated from *Chrysosporium lucknowense* that have neutral/alkaline cellulase activity.

The Examiner claims that an artisan would have been motivated at the time the invention was made to modify the teachings of Parslow to use cellulolytic enzymes from

*Chrysosporium lucknowense*. However, as the Examiner noted, Janeckova discloses only the isolation of *Chrysosporium lucknowense* from soil (Office Action – page 4) or more specifically the isolation of 37 species of soil fungi corresponding to fourteen genera, *i.e.*, *Absidia*, *Cunninghamella*, *Mucor*, *Rhizopus*, *Acremonium*, *Aspergillus*, *Fusarium*, *Geotrichum*, *Chrysosporium*, *Paecilomyces*, *Penicillium*, *Tolypocladium*, *Trichoderma* and *Verticillium* and not cellulolytic enzymes from *Chrysosporium lucknowense* that have neutral/alkaline cellulase activity. Furthermore, Janeckova clearly characterized *Chrysosporium lucknowense* as keratinophilic fungi (ABSTRACT), *i.e.*, ability to decompose keratinic substrates, such as  $\alpha$ -keratins, the insoluble fibrous proteins. An artisan could readily distinguish between the ability of a fungus to decompose cellulose (polysaccharides) and the ability of a fungus to decompose keratin (protein) and would not consider them to be the same or equivalent. Thus, based on the disclosure of Janeckova, one skilled in the art would not consider *Chrysosporium lucknowense* fungal species for their ability to decompose cellulose. Merely, the fact that Janeckova has isolated various fungal species from soil and one of them happened to be *Chrysosporium lucknowense* does not enable one skilled in the art to arrive at the claimed invention, *i.e.*, compositions comprising cellulases from *Chrysosporium lucknowense* that have neutral/alkaline cellulase activity. Currently, more than 70,000 species of fungi have been described; however, some estimates of total numbers suggest that 1.5 million species may exist (Hawksworth and Rossman, *Phytopathology* 87(9):888-891, 1997; respectfully submitted). Most fungi grow in terrestrial environments, and along with bacteria are the primary decomposers of organic matter in most if not all terrestrial ecosystems worldwide. Many fungal species have long been used as a source of food, a source for antibiotics and various enzymes. Thus, one skilled in the art would have performed great deal of undue experimentation to establish which of the fungi produces



cellulases that have neutral/alkaline cellulase activity and would not look to a limited disclosure in Janeckova to overcome the deficiencies of Parslow in order to arrive at the claimed invention. Thus, as demonstrated above, neither of the cited references discloses cellulolytic enzymes from *Chrysosporium lucknowense* that have neutral/alkaline cellulase activity. Therefore, the Examiner has used hindsight to suggest that an artisan at the time the invention was made would be motivated to combine the teachings of Parslow and Janeckova to use cellulolytic enzymes from *Chrysosporium lucknowense*, since neither Parslow, nor Janeckova disclose cellulases isolated from *Chrysosporium* species with neutral/alkaline cellulase activity.

Even though the Examiner points to Bukhtojarov for guidance to establish that the *Chrysosporium lucknowense* is able to produce various cellulases, applicants assert that this reference is inadmissible as evidence of knowledge in the art at the time the application was filed, *i.e.*, 1999, since it was published five years after the filing of the present application. One skilled in the art would not have the knowledge suggested by the Examiner to combine the teachings of Parslow and Janeckova without great deal of undue experimentation. Therefore, the rejection is improper in attempting to establish *prima facie* obviousness based on the disclosure after the filing date of the present application.

In light of the above arguments, applicants assert that neither the combination of, nor Parslow and Janeckova alone satisfy the components of the claimed composition. Janeckova does not remedy the deficiencies in the composition described by Parslow. Therefore, the combination of Parslow and Janeckova does not make obvious the claimed invention. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §103(a) rejection of claims 2-4, 6-65 and 80-83 in view of the aforementioned remarks.

Applicants respectfully submit that the invention as recited in the claims as presented herein is allowable over the art of record, and respectfully request that the respective rejections be withdrawn.

### CONCLUSION

Based on the foregoing amendments and remarks, Applicants respectfully request reconsideration and withdrawal of the rejection of claims and allowance of this application. Favorable action by the Examiner is earnestly solicited.

### AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for consideration of this Amendment to Deposit Account No. **13-4500**, Order No. 3123-4000US2.

In the event that an extension of time is required, or which may be required in addition to that requested in a petition for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for an extension of time to Deposit Account No. **13-4500**, Order No. 3123-4000US2.

Respectfully submitted,  
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# Where Are All the Undescribed Fungi?

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## ABSTRACT

Hawksworth, D. L., and Rossman, A. Y. 1997. Where are all the undescribed fungi? *Phytopathology* 87:888-891.

The hypothesis that there are 1.5 million fungal species on Earth, of which only about 70,000 are described, implies that 1.43 million remain undescribed. The recognition that many new species have yet to be found is of fundamental importance to plant pathologists, agronomists, and plant regulatory officials, among others, who continue to encounter diseases caused by previously unknown or understudied fungi. Unexplored habitats with their arsenal of unknown fungi are also of interest to those searching for novel organisms for use in biological control or for their pharmaceutical attributes. This paper presents data on the expected numbers of fungi in some relatively unexplored habitats, such as tropical forests, and those obligately associated with plants, lichens, and insects.

In addition to undiscovered species, many have been collected but remain lost or hidden as named species and ignored for lack of modern characterization; others have been collected and recognized as new species but remain undescribed. Some fungal species are unrecognized within erroneously circumscribed species, often based on presumed host specificity, while others exist as biological species but remain buried within those broadly defined species for lack of gross morphological characterization. From these data, one must conclude that enormous numbers of unrecognized fungi can be found almost everywhere, including one's own backyard.

*Additional keywords:* biodiversity, biosystematics, diversity, emerging diseases, inventory, lichenized fungi, plant pathogens.

The generally accepted estimate of the number of species of fungi on Earth is a conservative 1.5 million (17,19,25). This estimate was derived by extrapolating both from data on known fungi from well-studied regions, as well as data on well-studied fungi from plant hosts (17). Estimates of the species numbers in each major fungal group corroborate this conclusion (45). Although it is uncertain exactly how many fungal species are already known, one can be reasonably confident that it is in the range of 72,000 (21) to 100,000 (45). This implies that we know as little as 5%, i.e., only 1 in 20 of the species of fungi that exist. Clearly the knowledge gap is immense. But where are the remaining 1.43 million species to be found? The answer to this question is essential for mycologists and plant pathologists to be able to anticipate and respond to problems resulting from emerging diseases caused by previously unknown or understudied species and to prioritize and direct resources toward understanding the systematics of the most important and unknown groups of fungal plant pathogens. Additionally, the answer to this question will test the hypothesis itself and, thereby, address the skepticism of some nonmycologists about the number of undescribed species of fungi (16,39). In this paper, we draw attention to results that indicate where at least some of this enormous treasure-trove of fungi is to be found.

## TROPICAL FORESTS

Many biodiversity specialists, particularly those with expertise in macroorganisms, have determined that tropical forests are more species-rich than temperate forests; thus, this is generally assumed to be true for fungi as well. However, this hypothesis has not been rigorously tested for fungi, although a few comprehensive studies

have been undertaken that provide supportive evidence (8,19). For example, more than 500 fungi were identified in intensive studies of litter from only five tree species in Panama (11). After 1 year of intensive collecting in one forest in Kenya, 59 of 75 (79%) of the leaf-dwelling ascomycetes encountered were new species (40). Recent investigations of fungi on palms yielded 1,580 species, of which 75% of the species collected were new to science (27). Data on the primarily tropical ascomycete family Phyllostomataceae, tar leaf spot fungi, suggest that although 1,150 species have been described, the family may actually be support 169,000 species (7).

In exploring tropical regions for fungi, it is anticipated that the most widespread and common species in a site will tend to be found first. Most likely, such species have been collected before and are described already. However, the longer the time spent in intensive exploration, the more species are discovered, as evidenced by the continuing accounts of new species, primarily of mitotic fungi, that have been discovered in Taiwan during the past 16 years (29-37). A 2-month visit to Papua New Guinea yielded 6 genera and 89 species of lichen-forming and lichenicolous fungi new to science (2). Only through an intense examination of all fungi in a defined area will the finite number of fungal species be determined. The results of a recent workshop to plan an all-taxa biodiversity inventory in the Área de Conservación Guanacaste, Costa Rica, estimated the number of fungal species remaining to be encountered in the 120,000-ha reserve at around 50,000 (6).

An overall indication of whether the tropics are more species-rich in fungi than temperate regions might be expected based on an analysis of the origin of newly described fungi. The following data were extracted from the *Index of Fungi* database maintained at the International Mycological Institute (IMI), Egham, U.K. Tropical countries were the source of 49% of the 16,013 fungal species, including fossils, described as new during a 10-year period, 1981 to 1990. However, the United States was the origin of the largest number of new taxa, with 1,623 (10.1%) species, followed closely by India, with 1,554 (9.7%) species. Of the remaining countries with more than 1% of the total, 8 of 22 were tropical. Rather than indicating whether tropical countries are the richest

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source of new fungal species, these data suggest that all countries are inadequately known mycologically. Those yielding the most species new to science are merely the ones that receive the most intense scrutiny rather than those that are inherently the most species-rich. This view is supported by the situation in the British Isles, the most comprehensively studied region for fungi in the world. Here critical or intensive studies invariably yield species that have never been described: 460 species new to science were described from Great Britain and Ireland during the same 10-year period.

## UNEXPLORED HABITATS

The number of habitats that potentially support specialized and unique fungi is enormous. The fungi described as new to science during 1981 to 1990 (mentioned above) were associated with 1,982 host genera or substrata. Some previously unexplored substrata and habitats from which these fungi were found include the runens of herbivorous mammals; algae, lichens, and mosses; marine plants, including mangroves and driftwood, rocks; and insect scales. Soil and plant litter also continue to be a rich source of unusual fungi. Selected examples from a growing number of data sets are indicative of our current lack of knowledge.

### Plants

The 200,000 species of vascular plants continue to serve as the major reservoir of novel fungi. It has been estimated that there may be around 270,000 species of plant-pathogenic fungi in the tropics (47). As in the case of geographic analysis, it is intensity of study that best explains the observations. For example, during the 70-year period from 1920 to 1990, about three times as many new species of fungi were described from the relatively well-studied Poaceae compared to the related but less-studied Cyperaceae (8). Within the Poaceae, the number of fungi per potential host species ranged from a low of 0.3:1 in the genus *Poa* to 35:1 in the genus *Zea*, with 387 reported on *Zea mays* alone. Even among grasses in the United States, there are no reports of fungi on 45% of the grass hosts (15). After analyzing these data (15), Clay (10) reported that among the 20 grasses with the highest reported numbers of fungi in the United States, only 3 are native grasses. These numbers reflect the intensity of study, i.e., economic importance, rather than the biology of the host plants or the true fungal biodiversity of these two related plant families.

The same situation is found when one plant species becomes the focus of attention. In a search for potential fungal biocontrol agents, the pernicious woody shrub *Lantana camara* was scrutinized carefully and is now known to support 55 fungi, of which 28 appear to be restricted to that one host species (3). Three new species, one representing a new genus, were discovered during one study of *L. camara* in Brazil (3). The maritime rush *Juncus roemerianus*, found along the eastern coast of the United States, was not known to support any host-specific fungi until two researchers began investigating its mycota in 1991. From this one plant species, Kohlmeier et al. (28) have now described 20 new species, including 8 new genera and 1 new family. Even on this single-host plant, each of the numerous microhabitats are occupied by different fungal species.

In the tropics, it is particularly difficult to ascertain patterns of species richness. A. C. Batista and coworkers (12) recorded 3,340 fungi from Brazil associated with 523 plant species, equivalent to an average of 6.4 fungi per host plant studied. Such data were not collected for an analysis of species richness and are difficult to assess, because not all plants were studied and plant species without fungi were not listed. Further, although several new species could occur on a single leaf, no information is available from which to make sound extrapolations. How many leaves on the one tree species had novel fungi was not specifically reported nor was how many of those fungi occurred on different and even unrelated

trees nearby. Well-designed studies are needed to provide reliable data on this important issue.

Data on fungi reported on *Eucalyptus* recently have been compiled (46). Of the known 450 species in *Eucalyptus*, 1,350 species of fungi are known from 150 host species. The number of fungi from each host species ranges from 1 to 282. The highest number of fungi were found on *E. globulus*, and 150 of the 282 fungi on that host were not listed on any other species of *Eucalyptus*. Analyses of these and other data sets suggest that 5.3 unique fungi per host plant species is a reasonable working number (20).

### Lichens

Lichenicolous fungi, i.e., fungi growing on and in lichens, are a largely undiscovered group of organisms, many of which are novel genera and species. The number catalogued has increased from 457 species in 1976 to 894 in January 1996, a rise of 96% during 20 years. These totals are set to increase even more steeply, as evidenced by the following four studies. In a recent preliminary account of heterobasidiomycetes growing on lichens, 53 species were recognized, of which 46 (92%) were previously undescribed (13). An examination of fissuriculate pyrenomyces on tropical foliicolous lichens yielded 49 species, of which 36 (73%) were new to science (38). In examining fungi from the relatively well-studied lichen *Peltigera*, 96 species are now known, 61 of which occur only on this lichen host; 7 of these species were described only within the past year (22). A study of the fungi isolated from thalli of two fruticose lichen genera from one site in Germany revealed 506 strain types (42).

### Insects

In preparing estimates of the global numbers of fungi, Hawksworth (17) adopted a conservative approach to both insect numbers and the extent of host specificity of the insectivorous fungi. New data have been presented on the extent of host specificity in the insect-infecting Laboulbeniales, a group of fungi that occur on the exoskeletons of beetles and flies. Detailed comparisons were made between rich collections from sites in the United Kingdom and Sulawesi in Indonesia (50). This analysis suggests that, although only 1,855 species of Laboulbeniales are currently known, the actual number is likely to be between 10,000 and 50,000 species. This implies that only 3.7 to 18.5% of the species in the order are known. The situation may be even more extreme in the case of the Trichomyces, a group of enigmatic fungi occurring in the hindguts of insects and other invertebrates. These organisms are so poorly studied in most regions of the world that generalizations are hazardous. However, it is clear that these are a major source of undiscovered fungi even in the United Kingdom (41).

### New Techniques Applied to Previously Studied Substrata

Soil is one of the substrates from which fungi have been studied for many years, resulting in some relatively comprehensive accounts of soil fungi (14,44). Yet, the results of application of selective methods for isolating fungi from soil, such as selective isolation of low-temperature microfungi (9) or basidiomycetes (49), demonstrates that entire groups of fungi have been overlooked, even in relatively well-studied substrata. Species occurring at lower frequencies and those that are technically difficult to detect are less likely to have been discovered.

The increased use of techniques that favor detection of rare species, specifically particle filtration, has resulted in the isolation of several hundred fungi from one substrate without reaching an asymptote in the species-discovery curve (4). This technique, in which washed, pulverized, and filtered substrates are placed on a weak agar medium and closely observed, allows elimination of fast-growing fungi, so slower growing species can be detected. Using this technique, the number of fungal species isolated from the decaying plant parts of a single tropical plant exceeded 200

(4,43). In comparing species diversity from one plant host determined by two detection methods, the particle filtration technique yielded 10 times the number of fungal species detected by moist-chamber incubation (43). Thus, methods of isolation and observation play an important role in determining fungal diversity.

## LOST OR HIDDEN SPECIES

Many fungi are lost or hidden within existing constructs.

### Cryptic Biological Species

Fungi traditionally have been distinguished on the basis of morphological characters. However, genetic and molecular data suggest there are many functional biological species masquerading under a single species name or recognized only at an infraspecific rank or as a "special form." It is striking that almost every fungus critically studied by population biologists is found to comprise a number of reproductively isolated "biological species" (5). In distinguishing such biological species as formally described taxa, the biological information characteristics of that entity, e.g., its host range, pathogenicity, and growth conditions, likewise may be distinguished. If a biological species concept were adopted for all fungi, a case could be made for multiplying the existing total number of species in some groups by a factor of five or more.

### Lost Within Described Species

In some groups of fungi, there has been a tradition of describing species as new if the fungus is found on a new host plant. This can lead to the unnecessary proliferation of species names, but it also can mask situations in which more than one species of a fungal genus occurs on a single host. When critically examined, many of these fungi can be keyed out based on morphological criteria alone, without reference to the host. This was the case in a recent study on *Meliola* in Kenya (40). The net effect of this tradition is unknown, but experience at IMI suggests that in groups such as *Cercospora* and *Meliola* too few, rather than too many, species are currently being recognized. Recent work on species of *Alternaria* on *Citrus* exemplifies the situation in which isolates from one host are uncritically identified as a single, widely reported species considered to be host specific, while, in reality, a number of distinct fungal species occur on that host (48). Such simplifications have serious plant quarantine implications.

### Named and Orphaned Species

Many fungi have already been named but remain lost amongst the 250,000 species names that have not been characterized by modern standards. These species are not reported in the recent literature and are not included in the total number of known species. Many of these obscure names are probably synonyms of known species. Based on 15 fungal monographs (18), one can calculate a ratio of 2.5 synonyms for each "good" species. This ratio suggests that of the 250,000 species names in existence 100,000 of these are accepted species. Because only 72,000 are treated in the modern literature (21), there are 28,000 currently unadopted "orphan" species. Although this is not a major portion of the absentees, it does make the total number of expected species more realistic.

When genera are studied on a world scale, comprehensive monographs reveal spectacular numbers of previously undescribed species. Of 39 species accepted in *Meliolina* on tropical plants, 26 (67%) were new (26), and of 20 in *Lichenothelia* on rocks, 18 (90%) were new (24). In some cases, the number of species known in one fungal genus is reduced significantly when species prove to be plurivorous. This is exemplified by an account of *Didymosphaeria*, in which it was determined that the 7 accepted species had more than 100 synonyms (1). However, the usual pattern for little-studied fungi is that when investigated a large percentage of undescribed species are discovered.

## Collected and Unidentified

Most mycologists concentrate on one or two major groups of fungi that they collect and study throughout their lifetime. With the opportunity to visit previously unexplored regions of the world, each scientist bears the responsibility of collecting specimens of all groups of fungi and distributing them to experts. Yet, for many groups of fungi, including plant pathogens, no expert exists to study the specimens. At best, the specimens are deposited in the world's herbaria and identified only to the genus or not at all. Almost every mycological collection has folders, drawers, or boxes of specimens that represent a bonanza of as-yet unstudied and, possibly, undescribed species. Experts are needed who can undertake the rigorous study required to provide a formal description and place each new species in its taxonomic framework. In the case of major mycological institutions, these may amount to several thousand specimens and represent a significant portion of the undiscovered fungal biodiversity. To speculate, the number of undescribed fungal species represented by these specimens is probably more than 20,000.

## OPPORTUNITY AND CHALLENGE

The impression is often given that fungi in general have wider geographic distributions than vascular plants. In practice, the situation varies markedly between different groups. Particularly widespread are ancient fungi from phyla extending back to the first colonization of land in the Silurian region, some 300 million years before the first flowering plants. Fungi known from the early fossil record include endomycorrhizas, chytrids, myxomycetes, and some lichen-forming fungi. Also found in the category of fungi with wide geographic distributions are certain nonspecific, wood-rotting bracket fungi, opportunistic *r*-strategy spoilage molds, coprophilous fungi, and rapidly growing soil saprobes. Detailed comparisons between national checklists of fungi, although incomplete, would give some indication of the extent of overlap. However, when new species abound everywhere, it is difficult to know whether these discoveries are restricted, endemic species, or cosmopolitan taxa.

The extent of our ignorance poses major problems for mycologists and plant pathologists working in little-studied countries or habitats. What is already known? Which names are correct? How does one store information on new collections? How can one begin to identify newly collected specimens? How does one ascertain what is new? How does one gain access to available information when important publications no longer in print may be impossible to obtain? Conversely, how does one make new information accessible to others? These are complex problems that must be addressed (23). Answers are far from easy because of the lack of attention devoted to the myriad organisms involved.

Some may say that molecular approaches are the sole answer to describing fungal biodiversity. We do not. With around 1,800 fungi new to science being described every year, and not more than 100 species sequenced each year, the gap widens rather than narrows. Other than for fungi of medical and agricultural significance, the technological developments, including automated molecular and biochemical procedures, are unlikely to solve this problem within the foreseeable future. We suggest that those working in descriptive mycology must be well supported in the essential task of characterizing newly discovered fungal species. With increased electronic resources and use of the Internet to disseminate information, the products of every mycologist describing fungi can be made available rapidly along with user-friendly keys for identification linked to diagrams explaining cryptic features and illustrations of the species. Ideally, the two approaches to understanding fungal species would be combined, such that newly described species would be routinely sequenced and organisms representing the ends of molecular cladograms would be fully described. The result would be a whole that equaled more than the sum of its parts.

The short answer to the question posed in the title, "where are all the undescribed fungi?", is everywhere, including our own backyards. These fungi reside in unexplored niches as well as known habitats explored by applying something other than the usual isolation techniques. They exist in previously studied plants and soil in developed, temperate regions, as well as the ecologically diverse habitats of tropical regions. Recognizing the enormous undiscovered diversity in fungi, especially those associated with plants, soils, and insects in the tropics, is both an opportunity and a challenge. Researchers exploring this frontier are not surprised to uncover novel fungi that often represent new orders or families. A vast territory ripe for exploration awaits those who accept the exciting challenge of discovering the world's fungi.

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